GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2016), doi: 10.1111/gcbb.12319

Harvest management affects biomass composition responses of C4 perennial bioenergy grasses in the humid subtropical USA

CHAE-IN NA, JEFFREY R. FEDENKO, LYNN E. SOLLENBERGER and JOHN E. ERICKSON
Agronomy Department, University of Florida, Gainesville, FL 32611, USA

Abstract

Elephantgrass (*Pennisetum purpureum* Schum.) and energycane (*Saccharum* spp. hybrid) are high-yielding C4 grasses that are attractive biofuel feedstocks in the humid subtropics. Determining appropriate harvest management practices for optimal feedstock chemical composition is an important precursor to their successful use in production systems. In this research, we have investigated the effects of harvest timing and frequency on biomass nutrient, carbohydrate and lignin composition of UF1 and cv. Merkeron elephantgrasses and cv. L 79-1002 energycane. Biomass properties under increased harvest frequency (twice per year) and delayed harvest (once per year after frost) were compared with a control (once per year prior to frost). There were no differences between elephantgrass entries in structural carbohydrates; however, elephantgrasses had greater structural hexose concentration than energycane for single-harvest treatments (avg. 398 vs. 366 mg g^{-1}), a trait that is preferred for biofuel production. Delayed harvest of energycane decreased structural hexose compared with the control (374 vs. 357 mg g^{-1}) because nonstructural components accumulated in energycane stem as harvest was delayed. Frequent defoliation (2X) increased N, P, and ash concentrations (75% for N and P and 58% for ash) in harvested biomass compared with single-harvest treatments. We conclude that multiple harvests per year increase the harvest period during which feedstock is available for processing, but they do not result in optimal feedstock composition. In contrast, extending the period of feedstock supply by delaying a single harvest to after first freeze did not negatively affect cell wall constituent properties, while it increased length of the harvest period by ~30 days in the southeast USA.

Keywords: biofuel, elephantgrass, energycane, fiber composition, lignin, nitrogen, *Pennisetum purpureum*, *Saccharum* spp., structural carbohydrates

Received 10 September 2015 and accepted 26 October 2015

Introduction

Plant structural biomass consists primarily of three major types of polymers: cellulose, hemicellulose, and lignin. These polymers are strongly bonded by noncovalent forces and by covalent cross-linkages (Perez *et al.*, 2002). The cell wall is composed of long crystalline cellulose microfibrils embedded in a matrix of other polysaccharides (Perez *et al.*, 2002). In grasses, the predominant hemicellulosic polysaccharides in cell walls are glucuronoarabinoxylans that have a xylose backbone with arabinose and acetyl substitutions and constituents (Vermerris, 2008). Given the abundance of cellulose and hemicellulose in cell walls of perennial grasses, they represent a major source of structural carbohydrates for conversion to bioenergy. Lignin is the third major component of plant structural biomass and limits not only the physical accessibility of cellulose and hemicellulose, but also the activity of cellulolytic enzymes (Jung *et al.*, 2013). This occurs as a result of multiple factors, including shielding cellulose from microbial degradation by providing a surface that cellulolytic enzymes adsorb to irreversibly (Akin, 2007; Vermerris, 2008).

The proportions of cellulose and lignin in biomass affect the yield of biochemical conversion processes. For instance, due to the high lignin concentration in wood, more ethanol can be produced from switchgrass (*Panicum virgatum* L.) than that from the same weight of wood biomass (McKendry, 2002). Actual concentrations of monomers from cellulose and hemicellulose can be analyzed by procedures established by the National Renewable Energy Laboratory (NREL). These procedures allow for the quantification of sugar monomers from extractives (nonstructural carbohydrates or soluble sugars) and structural carbohydrates, and measurement of acid-soluble and insoluble lignin (Sluiter, 2008a,b).

Correspondence: Chae-In Na, tel. +1 352 392 3261, fax +1 352 392 1840, e-mail: dasan00@ufl.edu

The desired composition of biomass for bioenergy is dependent upon the postharvest conversion process used, and in most processes, lower levels of N and ash in the feedstock are preferred (Lewandowski & Heinz, 2003; Waramit *et al.*, 2011). High levels of N and/or ash can reduce thermochemical conversion efficiency and increase wear, while decreasing energy generation when used for co-firing (Shahandeh *et al.*, 2011). Perennial C4 grasses have up to two times greater N-use efficiency than C3 plants (Jakob *et al.*, 2009), and high biomass production with low tissue N concentration makes them important candidate bioenergy grasses.

Harvest management affects not only biomass yield but also its composition (Casler & Boe, 2003; Lewandowski & Heinz, 2003; Adler *et al.*, 2006). Miscanthus (*Miscanthus giganteus*) and giant reed (*Arundo donax* L.) showed a gradual decline in stem N concentration with increasing maturity in the United Kingdom (Smith & Slater, 2011). The decline in plant N concentration with increasing maturity has been associated with decreasing leaf proportion and much lower N concentration in stem than in leaves. For instance, average switchgrass leaf N concentration was 13.5 mg g^{-1} compared with 5.7 mg g^{-1} for stem (Shahandeh *et al.*, 2011). Similarly, giant reed and switchgrass leaves contained twofold or greater N when compared with the stem and showed a decline in N and P concentrations from October to December (Kering *et al.*, 2012).

Elephantgrass (or napiergrass; *Pennisetum purpureum* Schum.) is indigenous to sub-Saharan Africa in areas of rainfall exceeding 1000 mm. It is in the tribe Paniceae of the Poaceae family (Hanna *et al.*, 2004). Elephantgrass is a robust, creeping rhizomatous plant that perennates in the tropics and subtropics. Sollenberger *et al.* (2014) reported that elephantgrass breeding lines developed for biomass averaged 3.77 m in plant height, 271 g in tiller mass, and 7.4 mm in tiller diameter in Florida. Sugarcane (*Saccharum* spp.) hybrids have a high concentration of cellulose instead of sucrose, and they are a potentially valuable feedstock resource for cellulosic ethanol production. This is why high-cellulose *Saccharums* are called 'energycanes (*Saccharum* spp. hybrid)' (León *et al.*, 2010). To better understand *Saccharum* spp. as a biofuel crop, it is useful to note that there are three distinctive types. These include sugarcane (primarily sugar, conventional sugarcane), type I energycane (sugar and fiber), and type II energycane (primarily fiber).

Recent studies on harvest frequency and timing for elephantgrass and energycane grown in Florida, USA, found that two harvests annually negatively affected long-term biomass yield and plant persistence (Na *et al.*, 2015a,b). Harvest management is an important determinant of chemical composition of perennial grasses used for forage (Chaparro & Sollenberger, 1997), and it is reasonable to hypothesize that it affects composition of bioenergy feedstocks as well. Limited data exist describing harvest management effects on chemical composition of perennial grasses used for biofuel feedstocks grown on sandy soils in a subtropical environment. Therefore, the objective of this research was to determine the effect of harvest frequency and timing and grass entry on concentrations of plant structural and nonstructural carbohydrates, lignin, N, P, and ash in harvested biomass.

Materials and methods

Experimental site

The experiment was conducted during 2010 and 2011 at the Plant Science Research and Education Unit (PSREU) at Citra, FL (29.41°N, 82.17°W). The soil was a well-drained Candler sand (hyperthermic, uncoated Lamellic Quartzipsamments). Initial soil characterization (0–20 cm) showed an average soil pH of 7.0, and Mehlich-1 extractable P, K, and Mg of 54, 20, and 123 mg kg^{-1}, respectively. These concentrations are considered to be high for P, very low for K, and very high for Mg (Mylavarapu *et al.*, 2009). Monthly average, maximum, and minimum temperatures (Fig. 1) and monthly precipitation (Fig. 2) are shown for the experimental period.

Treatments and experimental design

Treatments included all factorial combinations of three grass entries and three harvest management practices. Each treatment was replicated four times in a split-plot arrangement of a randomized complete block design. Harvest treatment was the main plot and grass entry was the subplot. The three grass entries included two elephantgrasses, cv. Merkeron (Burton, 1989) and a breeding line referred to as UF1, and cv. L 79-1002 type II energycane (Bischoff *et al.*, 2008). These two species were chosen because earlier work with biomass feedstocks identified them as having the greatest potential in this region (Woodard & Prine, 1993). Merkeron and L 79-1002 were also chosen because they were the cultivars with the largest current presence in the region. Breeding line UF1 was included because previous research had demonstrated its high yield potential and preferred morphological characteristics (Sollenberger *et al.*, 2014; Na *et al.*, 2015a,b).

Three harvest management treatments were implemented that included different harvest frequency and timing. These were (i) two harvests per year (late July, named 2X-July; November harvest of regrowth after 2X-July harvest, named 2X-Nov), (ii) one harvest per year in fall [at initiation of Merkeron flowering (first entry to flower) and before first freeze; named 1X-Nov], and (iii) one harvest per year in winter (within 1 week following first freeze, with a freeze defined as a temperature of less than 0 °C at 2 m above soil level resulting in

 doi: 10.1111/gcbb.12319

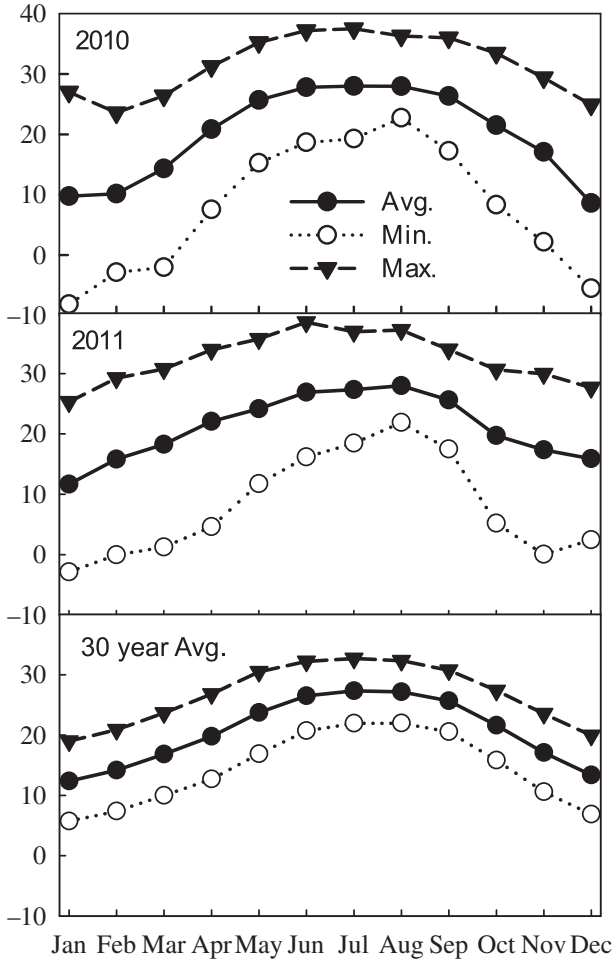

Fig. 1 Monthly average and monthly maximum and minimum air temperatures for 2010 and 2011 for the experimental location (available at Florida Automated Weather Network, http://fawn.ifas.ufl.edu), and the 30-year average for Gainesville, Florida (available at Florida Climate Center, http://climatecenter.fsu.edu).

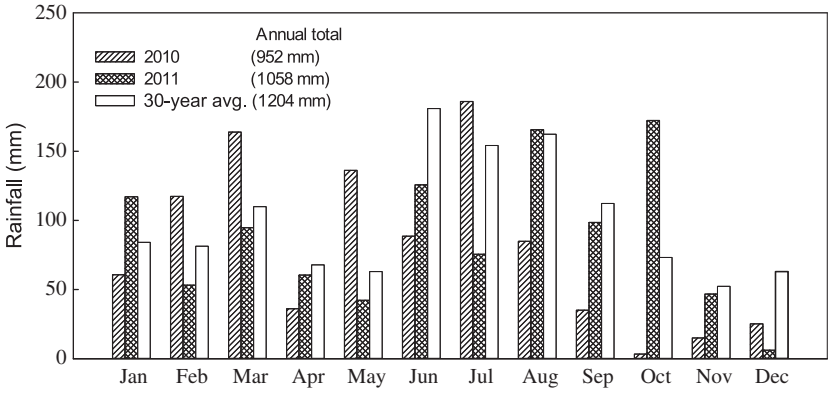

Fig. 2 Monthly rainfall for 2010 and 2011 for the experimental location (available at Florida Automated Weather Network, http://fawn.ifas.ufl.edu), and the 30-year average for Gainesville, Florida (available at Florida Climate Center, http://climatecenter.fsu.edu).

complete kill of leaves, or at full flowering of Merkeron, whichever happened first; named 1X-Dec). In 2010, harvests occurred on 30 July for 2X-July, 10 November for 1X-Nov and 2X-Nov, and 9 December for 1X-Dec. In 2011, harvest dates were 21 July for 2X-July, 8 November for 1X-Nov and 2X-Nov, and 15 December for 1X-Dec.

 doi: 10.1111/gcbb.12319

Plot preparation and management

Plots contained six rows, each 6 m long, with 1 m spacing between rows, and were established using aboveground stem pieces planted on December 15, 2009. Thus, the 2010 data are from the establishment year. In both years, N was applied as ammonium sulfate [$(NH_4)_2SO_4$] at a rate of 150 kg N ha^{-1}, and K was applied as muriate of potash (KCl) at a rate of 90 kg K ha^{-1}. Nutrients were split applied, with applications of 50 kg N and 45 kg K ha^{-1} in mid-April and 100 kg N and 45 kg K ha^{-1} in mid-May. No P was applied based on soil test results indicating sufficient levels. Limited irrigation was applied to the experiment only at visual signs of significant drought stress, for example, severe leaf rolling. There were five irrigation events in 2010 (total of 60 mm) and three irrigation events in 2011 (total of 50 mm) using a traveling gun irrigation system.

At harvest, four representative tillers (approximately 3 kg fresh weight) were selected from mid-row at least a meter from all borders for determination of plant part proportion, dry matter, and chemical composition of harvested feedstock. Tillers were hand-separated into leaf (blade and sheath) and stem (including inflorescence, if present) components. Leaf : stem ratio data were reported previously (Na *et al.*, 2015b). Samples were dried at 60 °C until constant weight. Stem samples were initially grounded through a hammer mill to reduce particle size. Stem and leaf samples were ground to pass a 1-mm stainless steel screen in a Wiley mill (Model 4 Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA). Ground samples were transferred to an airtight container immediately.

Biomass fiber analysis

Each component of dried biomass (stem and leaves) was analyzed for chemical composition. A modified NREL procedure was used for compositional analysis (Sluiter, 2008a,b). In the modified NREL procedure, dried biomass was analyzed for nonstructural extractives, structural carbohydrates, and total lignin (Fedenko *et al.*, 2013). For nonstructural extractives, 100 ml of deionized (D.I.) water was added to 1 g of dried sample and autoclaved in sealed pressure tubes (ACE Glass, Inc., Vineland, NJ, USA) at 121 °C for 1 h. Samples were then vacuum-filtered through wet-strengthened 113 filter paper (Whatman, GE healthcare, Little Chalfont, UK). Filtered extractives were collected for nonstructural carbohydrate analysis. The captured structural biomass from extraction was dried at 40 °C and then weighed. A 0.3-g dry subsample was used for two-stage acid hydrolysis; the first stage was a 1-h incubation at 30 °C with concentrated sulfuric acid (72%, Fluka Alalytical; Sigma-Aldrich, St. Louis, MO, USA). In the second stage, sulfuric acid was diluted to 4% by adding D.I. water for 1-h digestion under elevated pressure and temperature (121 °C and 103 kPa). Hydrolyzed samples were vacuum-filtered through a medium-porosity filtering crucible (Coors #60531; CoorsTek, Golden, CO, USA). Two aliquots of filtrate were taken, with one analyzed in duplicate for acid-soluble lignin at a wavelength of 240 nm using a UV–vis spectrophotometer (StellarNet, Inc., Tampa, FL, USA). The second aliquot collected from hydrolyzed samples was neutralized to pH 5–7 using calcium carbonate. Insoluble lignin was determined gravimetrically as total solids remaining in the crucible after vacuum filtration of hydrolyzed biomass. Total lignin was calculated as the sum of acid-soluble and insoluble lignin. Extractives and neutralized hydrolyzed samples were filtered through a 0.22-μm syringe filter and analyzed by HPLC (Perkin-Elmer Flexar system, Waltham, MA, USA) using a refractive index detector and a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) maintained at 50 °C. Sulfuric acid (HPLC grade, 4 mM) was used as the mobile phase at a flow rate of 0.4 ml min^{-1} with a 10-μl injection and 40-min run time. Perkin-Elmer's Chromera software was used to quantify peaks (Fedenko, 2011).

Extractives are considered to be the sum of all nonstructural components of plant tissue removed during extraction, of which soluble sugars are a major component. They were determined on a mass balance basis as the difference between initial sample weight and sample weight after hot water extraction (Sluiter, 2008a). Hexoses and pentoses are the sum of six-carbon (6C; glucose and mannose) and five-carbon (5C; xylose and arabinose) structural carbohydrates, respectively. Mannose was never present at minimum quantifiable levels in this study. Total lignin is the sum of acid-soluble and insoluble lignin.

Total nitrogen, phosphorus, and ash

For N and P analyses, a modified Kjeldahl procedure was conducted. Samples were digested using a modification of the aluminum block digestion procedure (Gallaher *et al.*, 1975). Sample weight was 0.25 g, catalyst used was 1.5 g of 9 : 1 K_2SO_4 : $CuSO_4$, and digestion was conducted for at least 4 h at 375 °C using 6 ml of sulfuric acid and 2 ml hydrogen peroxide. Analysis of digestate was carried out using the Technicon Autoanalyzer and semiautomated colorimetry to determine N and P in the digestate (Hambleton, 1977). Absolute dry matter was determined by oven drying at 105 °C to constant weight and then samples were ashed at 500 °C for 6 h to determine ash concentration.

Statistical analysis

Data were analyzed using mixed-model methods in PROC MIXED (SAS Institute, 2008). In all models, harvest treatment and grass entry were considered fixed effects. Year was considered a repeated measurement (fixed). Block and interactions with block were considered random effects. Because there were two harvests per year in the 2X treatment, samples from both harvests were analyzed separately in the laboratory, and the model effectively contains four levels of the harvest management treatment, that is, 2X-July, 2X-Nov, 1X-Nov, and 1X-Dec. Leaf, stem, and total aboveground biomass data were analyzed separately for each factor. Total aboveground biomass data were calculated as the weighted average of leaf and stem composition for each factor. Means were compared using the pdiff test of LSMEANS. All means reported in the text are least-square means and were considered different if $P \leq 0.05$. Because the effects of greatest interest were harvest management, entry, and their interaction, and because the harvest management × entry interaction was significant in most cases, data presented are the means for this interaction. If the

 doi: 10.1111/gcbb.12319

interaction was not significant, only main effect means were compared.

Results

Extractives and total soluble sugars

A harvest management × entry interaction occurred ($P = 0.044$) for leaf extractives because UF1 elephantgrass showed a greater concentration than other entries in the 1X-Dec harvest, but there were no differences between elephantgrass entries for the other harvest treatments (Table 1). Single-harvest (1X-Nov and 1X-Dec) leaf-extractive concentrations were lower than either 2X-July or 2X-Nov for all grass entries. A harvest management x entry interaction occurred for stem extractives ($P < 0.001$, Table 1). L 79-1002 stem extractives were greatest in 1X-Dec (338 mg g^{-1}) followed by 1X-Nov (313 mg g^{-1}), while elephantgrass entries had greater stem extractives in 2X-July and 2X-Nov treatments (avg. 275 and 268 mg g^{-1}, respectively) than 1X-Nov and 1X-Dec (avg. 256 and 252 mg g^{-1}, respectively). In 1X-Dec, L 79-1002 showed the greatest stem extractive concentration followed by UF1 (263 mg g^{-1}) and then Merkeron (241 mg g^{-1}). There also was a harvest management x entry interaction ($P < 0.001$) for extractive concentration in total aboveground biomass. There were no differences among entries within the 2X-Nov harvest, but L 79-1002 had greater extractive concentration than Merkeron for all other harvest treatments and greater than UF1 in both 1X-Nov and 1X-Dec.

Total soluble sugars in leaf tissue extractives showed a harvest management × entry interaction ($P = 0.044$) because there were no differences among entries within the 2X-July management, but differences among entries occurred for other harvest managements. One of the elephantgrasses always had the greatest concentration, except for 2X-July, when there were no differences among entries (Table 2). In the stem, a harvest management × entry interaction occurred ($P < 0.001$) for total soluble sugar concentrations (Table 2). For all three entries, 2X-July total soluble sugar concentration was

Table 1 Effect of grass entry and harvest management interaction on extractives in leaf ($P = 0.044$), stem ($P < 0.001$), and total above-ground biomass ($P < 0.001$). Data are means across four replicates and 2 years ($n = 8$)

	Harvest management*			
	2X-July	2X-Nov	1X-Nov	1X-Dec
Entry	mg extractives g^{-1} dry matter			
Leaf				
L 79-1002	199 a†B‡	196 aB	164 bB	173 bC
Merkeron	221 aA	226 aA	194 bA	187 bB
UF1	216 aA	225 aA	197 bA	201 bA
SE	4.0			
Stem				
L 79-1002	302 bcA	289 cA	313 bA	338 aA
Merkeron	265 aC	262 aC	250 abC	241 bC
UF1	285 aB	274 abB	262 bB	263 bB
SE	7.9			
Total				
L 79-1002	267 bA	251 cA	269 bA	289 aA
Merkeron	251 aB	248 abA	234 bcB	229 cC
UF1	262 aA	256 abA	244 bB	254 abB
SE	6.0			

*Harvest management treatments were harvested twice per year in July and November (2X-July and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Harvest management means within an entry not followed by the same lower case letter are different ($P < 0.05$).

‡Entry means within a harvest management not followed by the same upper case letter are different ($P < 0.05$).

Table 2 Effect of grass entry and harvest management interaction on total soluble sugars concentration in leaf ($P = 0.044$), stem ($P < 0.001$), and total above-ground biomass ($P < 0.001$). Data are means across four replicates and 2 years ($n = 8$)

	Harvest management*			
	2X-July	2X-Nov	1X-Nov	1X-Dec
Entry	mg total soluble sugars g^{-1} dry matter			
Leaf				
L 79-1002	23 b†A‡	32 aB	14 cB	22 bB
Merkeron	26 bA	40 aA	24 bA	22 bB
UF1	26 bA	35 aAB	29 abA	33 abA
SE	2.9			
Stem				
L 79-1002	152 cA	165 cA	228 bA	280 aA
Merkeron	97 bB	134 aB	138 aC	142 aC
UF1	138 bA	147 bB	161 bB	189 aB
SE	11.2			
Total				
L 79-1002	109 cA	110 cA	163 bA	205 aA
Merkeron	74 cB	96 bB	106 abC	116 aC
UF1	102 cA	106 cAB	126 bB	167 aB
SE	6.9			

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Harvest management means within an entry not followed by the same lower case letter are different ($P < 0.05$).

‡Entry means within a harvest management not followed by the same upper case letter are different ($P < 0.05$).

 doi: 10.1111/gcbb.12319

less than 1X-Dec. The magnitude of this difference varied among entries (84% increase in L 79-1002, 51% in Merkeron, and 36% in UF1, respectively). Across treatments, total soluble sugars in the leaves were several times lower than that in the stem (avg. 27 vs. 164 mg soluble sugars g^{-1}). In total aboveground biomass, there was a harvest management x entry interaction for soluble sugars (Table 2, $P < 0.001$). Because the concentration of leaf soluble sugar was very small and because leaves composed a small percentage of harvested biomass, particularly for 1X-Nov and 1X-Dec, total aboveground biomass soluble sugar concentrations generally followed the same pattern as stem. Concentration of total soluble sugars varied mostly among entries for 1X vs. 2X harvest treatments. Interestingly, among elephantgrass entries, UF1 showed 44% greater soluble sugar concentration in total aboveground biomass compared with Merkeron. Further investigation of UF1 soluble sugar concentration is needed to determine the specific sugars present.

Structural hexose and pentose

In the leaves, structural hexose concentration was affected by harvest management and entry ($P < 0.001$ for both). Entry L 79-1002 showed greater hexose concentration than elephantgrass entries by 7% (Table 3). Structural hexose concentrations were greater in 1X-Nov and 1X-Dec followed by 2X-July and then 2X-Nov. In stem, there was a harvest management × entry interaction ($P = 0.001$). Stems of elephantgrass entries had greater structural hexose concentration than those of L 79-1002 for all harvest treatments (Table 3). Structural hexose concentration in L 79-1002 was similar for 2X and 1X-Nov treatments, but it was least for 1X-Dec. Merkeron and UF1 had lowest structural hexose concentration in 2X-Nov, while UF1 had greater hexose concentration in 1X-Nov and then 1X-Dec, but for Merkeron, the 1X-Nov and 1X-Dec treatments were not different.

In total aboveground biomass, harvest management x entry interaction occurred ($P = 0.001$). For 2X-Nov, there were no differences among grass entries; however, entry L 79-1002 had lower structural hexose concentration than the elephantgrasses in 2X-July, 1X-Nov, and 1X-Dec (Table 3). Comparing harvest managements within an entry, both Merkeron and UF1 had greatest hexose concentration in 1X-Nov and 1X-Dec and least in 2X-Nov, while energycane hexose concentration varied relatively little and was greater in 1X-Nov than in 2X-Nov and 1X-Dec.

Concentration of structural pentose in leaves was affected by harvest management ($P = 0.001$). The 2X-July treatment had the lowest pentose concentration compared with other treatments, but there were no differences among the other harvests. There was harvest management x entry interaction for stem pentose concentration ($P = 0.003$). The most pronounced difference was that 2X-Nov had the greatest pentose concentration among all entries. For both 2X treatments, L 79-1002 had the greatest pentose concentration compared with the two elephantgrasses (Table 4). There were no differences among entries for the 1X-Nov harvest, while for 1X-Dec, Merkeron had greater pentose concentration than L 79-1002. In total aboveground biomass, structural pentose concentration was affected by harvest management and entry ($P = 0.001$, $P < 0.001$, respectively). Entry L 79-1002 had greater pentose concentration than the elephantgrasses; however, the differences among entries were relatively small (4–5 mg of pentose g^{-1}). The 2X-Nov harvest management had greater pentose concentration than the other defoliation treatments.

Table 3 Effect of grass entry and harvest management interaction on structural hexose concentration in leaf ($P = 0.562$), stem ($P = 0.001$), and total above-ground biomass ($P = 0.001$). Data are means across four replicates and 2 years ($n = 8$)

	Harvest management*				
	2X-July	2X-Nov	1X-Nov	1X-Dec	Mean
Entry	mg hexose g^{-1} dry matter				
Leaf					
L 79-1002	371	356	404	395	381 A†
Merkeron	346	334	370	369	355 B
UF1	342	333	369	357	350 B
Mean	353 b‡	341 c	381 a	374 a	
SE	5.2				
Stem					
L 79-1002	363 a§B¶	361 aB	361 aB	342 bB	
Merkeron	400 bA	385 cA	415 aA	406 abA	
UF1	397 bA	378 cA	413 aA	394 bA	
SE	7.5				
Total					
L 79-1002	367 ab§B¶	360 bA	374 aB	357 bB	
Merkeron	382 bA	365 cA	403 aA	399 aA	
UF1	381 bA	362 cA	402 aA	389 bA	
SE	6.2				

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

‡Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).

§Harvest management means within an entry not followed by the same lower case letter are different ($P < 0.05$).

¶Entry means within a harvest management not followed by the same upper case letter are different ($P < 0.05$).

Table 4 Effect of grass entry and harvest management interaction on structural pentose concentration in leaf ($P = 0.977$), stem ($P = 0.003$), and total above-ground biomass ($P = 0.139$). Data are means across four replicates and 2 years ($n = 8$)

	Harvest management*				
	2X-July	2X-Nov	1X-Nov	1X-Dec	Mean
Entry	mg pentose g^{-1} dry matter				
Leaf					
L 79-1002	252	266	273	272	266A
Merkeron	251	266	271	272	265A
UF1	254	273	279	274	270A
Mean	252 b†	268 a	274 a	273 a	
SE	4.0				
Stem					
L 79-1002	217 b‡A§	244 aA	209 bcA	202 cB	
Merkeron	206 bB	233 aB	209 bA	213 bA	
UF1	206 bB	230 aB	209 bA	209 bAB	
SE	3.1				
Total					
L 79-1002	229	253	228	222	233 A¶
Merkeron	221	246	225	225	229 B
UF1	222	246	228	218	228 B
Mean	224 b‡	248 a	227 b	222 b	
SE	2.9				

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).

‡Harvest management means within an entry not followed by the same lower case letter are different ($P < 0.05$).

§Entry means within a harvest management not followed by the same upper case letter are different ($P < 0.05$).

¶Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

Lignin

Leaf lignin concentration was affected by harvest management ($P = 0.005$) and entry ($P < 0.001$). Entry L 79-1002 leaf lignin concentration was greater than in Merkeron and UF1 by 7–8% (218, 204, and 201 mg lignin g^{-1}, respectively, Table 5). Single-harvest management (1X-Nov and 1X-Dec; 214 and 212 mg lignin g^{-1}, respectively) had greater leaf lignin concentration than 2X harvest managements (2X-July and 2X-Nov; 204 and 202 mg lignin g^{-1}, respectively). For stem lignin concentration, there was a harvest management × entry interaction ($P = 0.002$). For all entries, 1X-Nov and 1X-Dec biomass had greater stem lignin concentration than either of the 2X harvest managements (Table 5). The interaction occurred because there were no differences between Merkeron and UF1 for 1X-Nov; in contrast, within the other harvest management treatments, Merkeron had greater stem lignin concentration than UF1 which had a greater lignin concentration than L 79-1002. In total aboveground biomass, a harvest management x entry interaction occurred ($P = 0.002$). Similar to stem lignin concentration, both the 2X harvest managements generally had lesser lignin concentration compared with 1X-Nov and 1X-Dec. Merkeron generally had greater lignin concentration than energycane.

Nitrogen, phosphorus, and ash

Nitrogen concentration in the leaves was affected by harvest management and entry ($P < 0.001$ for both). Energycane leaves had a lower N concentration (9.9 mg g^{-1}) than elephantgrass entries (average of 11.5 mg g^{-1}, Table 6). Leaf N concentration averaged approximately 37% lower for the single-harvest treat-

Table 5 Effect of grass entry and harvest management interaction on lignin concentration in leaf ($P = 0.069$), stem ($P = 0.002$), and total above-ground biomass ($P = 0.002$). Data are means across four replicates and 2 years ($n = 8$)

	Harvest management*				
	2X-July	2X-Nov	1X-Nov	1X-Dec	Mean
Entry	mg lignin g^{-1} dry matter				
Leaf					
L 79-1002	213	213	226	221	218 A†
Merkeron	199	197	210	211	204 B
UF1	200	197	204	202	201 C
Mean	204 b‡	202 b	214 a	212 a	
SE	2.8				
Stem					
L 79-1002	167 b§C¶	167 bC	182 aB	177 aC	
Merkeron	184 bA	182 bA	203 aA	206 aA	
UF1	176 bB	174 bB	199 aA	199 aB	
SE	3.5				
Total					
L 79-1002	183 c§B¶	186 bcAB	195 aB	190 abC	
Merkeron	189 bA	188 bA	204 aA	207 aA	
UF1	183 bB	183 bB	201 aA	200 aB	
SE	2.9				

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

‡Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).

§Harvest management means within an entry not followed by the same lower case letter are different ($P < 0.05$).

¶Entry means within a harvest management not followed by the same upper case letter are different ($P < 0.05$).

 doi: 10.1111/gcbb.12319

Table 6 Effect of grass entry and harvest management interaction on nitrogen concentration in leaf ($P = 0.334$), stem ($P = 0.942$), and total above-ground biomass ($P = 0.955$). Data are means across four replicates and 2 years ($n = 8$)

	Harvest management*				
	2X-July	2X-Nov	1X-Nov	1X-Dec	Mean
Entry	mg nitrogen g^{-1} dry matter				
Leaf					
L 79-1002	12.5	12.9	7.4	6.6	9.9 B†
Merkeron	14.1	14.2	9.5	8.2	11.5 A
UF1	13.0	13.7	9.5	9.5	11.4 A
Mean	13.2 a‡	13.6 a	8.8 b	8.1 b	
SE	0.76				
Stem					
L 79-1002	5.3	4.4	3.3	3.0	4.0 B
Merkeron	7.0	5.2	4.4	3.9	5.1 A
UF1	5.5	4.9	3.4	3.4	4.3 B
Mean	5.9 a	4.9 b	3.7 c	3.5 c	
SE	0.56				
Total					
L 79-1002	7.8	7.9	4.8	4.1	6.1 B
Merkeron	9.1	8.7	5.8	4.8	7.1 A
UF1	7.6	8.0	4.6	4.2	6.1 B
Mean	8.2 a	8.2 a	5.0 b	4.4 b	
SE	0.58				

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

‡Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).

ments (1X-Nov and 1X-Dec) compared with the 2X treatments.

In stem, N concentration was affected by harvest management ($P = 0.002$) and entry ($P = 0.011$). Merkeron (5.1 mg g^{-1}) N concentration was greater than other entries (4.3 mg g^{-1} in UF1 and 4.0 mg g^{-1} in L 79-1002, Table 6). The 2X-July treatment, which was harvested about 8 week after the final spring fertilizer application, had greatest N concentration, while 1X-Nov and 1X-Dec harvest management were lowest in N. Similar to stem N, total aboveground biomass N concentration was affected by harvest management ($P < 0.001$) and entry ($P = 0.004$). Merkeron had greatest N concentration. Nitrogen concentration decreased by 39% from 2X-July and 2X-Nov to 1X-Nov and 46% to 1X-Dec.

Phosphorus concentration in leaves, stem, and total aboveground biomass showed similar trends to N concentrations. Phosphorus concentration was affected by harvest management and entry in leaf ($P = 0.002$ and 0.001, respectively), stem ($P < 0.001$ for both), and total aboveground biomass ($P = 0.001$ and <0.001, respectively). In both plant parts and in total biomass, Merkeron had the greatest overall P concentration (Table 7). In leaves, stem, and total plant biomass, P concentration was greater for the 2X treatments compared with 1X-Nov and 1X-Dec. For example, in total aboveground biomass, P concentration in 2X-Nov was approximately twice as great as the single-harvest treatment.

There were harvest management and entry effects on leaf ash concentration ($P = 0.012$ and <0.001, respectively). Entry UF1 had the greatest leaf ash concentration followed by Merkeron and then L 79-1002 (Table 8). The 1X-Nov and 1X-Dec harvested biomass had lesser ash concentration (48 and 44 mg g^{-1}, respectively) than 2X-July and 2X-Nov treatments (54 mg g^{-1} for both). In stem, ash concentration was affected by harvest management ($P < 0.001$). The average of the sin-

Table 7 Effect of grass entry harvest and management interaction on phosphorus concentration in leaf ($P = 0.139$), stem ($P = 0.360$), and total above-ground biomass ($P = 0.348$). Data are means across four replicates and 2 years ($n = 8$)

	Harvest management*				
	2X-July	2X-Nov	1X-Nov	1X-Dec	Mean
Entry	mg phosphorus g^{-1} dry matter				
Leaf					
L 79-1002	1.40	1.94	0.72	0.57	1.16 B†
Merkeron	1.95	2.13	1.14	1.03	1.56 A
UF1	1.32	1.51	0.88	1.00	1.17 B
Mean	1.56 a‡	1.86 a	0.91 b	0.87 b	
SE	0.18				
Stem					
L 79-1002	1.30	1.44	0.70	0.63	1.02 B
Merkeron	1.64	1.99	1.21	1.09	1.48 A
UF1	1.07	1.56	0.78	0.94	1.09 B
Mean	1.34 b	1.66 a	0.89 c	0.89 c	
SE	0.14				
Total					
L 79-1002	1.31	1.66	0.73	0.62	1.08 B
Merkeron	1.71	2.04	1.19	1.07	1.50 A
UF1	1.13	1.53	0.80	0.95	1.10 B
Mean	1.38 b	1.74 a	0.90 c	0.88 c	
SE	0.15				

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

‡Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).

Table 8 Effect of grass entry and harvest management interaction on ash concentration in leaf ($P = 0.359$), stem ($P = 0.257$), and total above-ground biomass ($P = 0.853$). Data are means across four replicates and 2 years ($n = 8$)

	Harvest management*				
	2X-July	2X-Nov	1X-Nov	1X-Dec	Mean
Entry	mg ash g^{-1} dry matter				
Leaf					
L 79-1002	44	45	33	32	38 C†
Merkeron	59	57	50	43	52 B
UF1	60	60	60	56	59 A
Mean	54 a‡	54 ab	48 bc	44 c	
SE	3.9				
Stem					
L 79-1002	44	43	28	22	34 A
Merkeron	40	43	26	25	34 A
UF1	39	46	25	27	34 A
Mean	41 a	44 a	26 b	24 b	
SE	2.5				
Total					
L 79-1002	44	44	29	24	35 B
Merkeron	46	48	32	29	39 A
UF1	45	51	32	31	40 A
Mean	45 a	48 a	31 b	28 b	
SE	2.4				

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

‡Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).

gle-harvest treatments (1X-Nov and 1X-Dec) was 41% less than the average of 2X-July and 2X-Nov (Table 8). In total aboveground biomass, ash concentration was affected by harvest management and entry ($P < 0.001$, <0.012, respectively). Elephantgrass entries had greater (up to 11%) ash concentration than energycane (Table 8). Because of generally greater ash concentration in leaves than stem and leaf senescence over the season, total ash concentration in 1X-Nov and 1X-Dec was less than in either of the 2X treatments.

Discussion

Extractives and total soluble sugars

Elephantgrass and energycane extractive concentration responses to harvest management were different. Elephantgrass biomass had greatest extractives concentrations when harvested twice per year, while energycane extractives were greatest when biomass was harvested once per year. This response was due to accumulation of extractives in energycane stems late in the growing season. Because of the preponderance of stem in harvested biomass, especially when harvested once per year, the effects of leaf extractives concentration were small. There are very limited data available for extractives concentration in perennial C4 grasses. An elephantgrass study in Vietnam reported 182 mg g^{-1} of extractives measured using a hot water extraction (Hoa *et al*., 2008). As observed in the present study, Fedenko *et al*. (2013) found that total extractives concentration was greater for energycane than elephantgrass when harvested once per year in fall prior to a freeze event (avg. 234 vs. 188 mg g^{-1}) due to sugar accumulation in energycane. In Colorado, extractives concentration varied among entries including 164 mg g^{-1} for the C4 perennial switchgrass, 254 mg g^{-1} for the C3 perennial grass tall fescue (*Festuca arundinacea* L.), and 172 mg g^{-1} for maize stover (*Zea may* L.; Thammasouk *et al*., 1997).

Stem was the major contributor of soluble sugars to total aboveground biomass for both grass species in the present study. Although differences existed in soluble sugar concentration in leaves, they contributed marginally to differences on a total harvested biomass basis because leaf proportion was low and leaf abscission occurred late in the growing season (Na *et al*., 2015b). Single-harvest (1X-Nov and Dec) energycane showed greatest soluble sugar concentration in total harvested biomass because of greater accumulation in the stem, mostly of sucrose. Although energycane stem has a relatively low sugar concentration compared with sugarcane (*Saccharum* spp. hybrid), it shows measurable levels of soluble sugar accumulation late in the season (Bischoff *et al*., 2008; Kim & Day, 2011; Tew *et al*., 2011). Unlike structural carbohydrates, non-cell wall carbohydrates are directly fermentable; however, they are susceptible to microbial degradation during storage (Dien *et al*., 2006). Moreover, by degradation of free sugars, cellulosic fermentation inhibitors including furfural can be produced (Tran & Chambers, 1986; Zhang *et al*., 2011). If they cannot be used for conversion to biofuel, they will decrease feedstock quality as they reduce concentration of structural carbohydrates proportionally. In the previous research, L 79-1002 had 101% (first year) and 45% (second year) greater soluble sugar concentration than Merkeron (Fedenko *et al*., 2013), a similar result to the present study.

Structural hexose and pentose

For lignocellulosic biomass, ethanol fermentation efficiency is determined by the quality of structural carbohydrates (Lu & Mosier, 2008), so it is critical to investigate tissue structural carbohydrates. Energycane

 doi: 10.1111/gcbb.12319

had greater leaf structural hexose concentration than elephantgrass entries; however, elephantgrass entries showed greater hexose concentration in both stem and total aboveground biomass than energycane. This was due at least in part to an increase in total soluble sugar over time in energycane stem tissue, which resulted in a dilution of the concentration of structural components. A large amount of glucose from cell wall structural components is advantageous for ethanol production because glucose can be converted by most organisms more efficiently to ethanol than most other sugars, especially pentose sugars (Lu & Mosier, 2008). Similar to values observed in the present study, structural hexose concentrations of 347 mg g^{-1} have been reported for mixtures of C4 grasses in Minnesota, USA (Gillitzer *et al*., 2013). Chemical composition of six-carbon structural sugars was 300–337 mg g^{-1} for switchgrass (Xu *et al*., 2011). In the prior work at the location of the present study, structural glucose concentration in elephantgrass and energycane was 374 vs. 366 mg g^{-1} in the first year and 448 vs. 432 mg g^{-1} in the second year, respectively (Fedenko *et al*., 2013). Late harvests and increasing maturity have been observed to increase glucose and nonglucose structural sugars in reed canarygrass (*Phalaris arundinacea* L.) (233–286 mg g^{-1}) and switchgrass (294–340 mg g^{-1}) (Dien *et al*., 2006). This corresponds to what was observed for elephantgrass, but not for energycane, in the present study.

Unlike structural hexose results, energycane showed the greatest pentose concentration in total aboveground biomass. As pentose concentrations increased, structural hexose concentrations decreased in the 2X-Nov harvest for total biomass. As indicated earlier, because of the fermentation inefficiency of pentose by most microorganisms, it is less favorable than hexose (Lu & Mosier, 2008). In switchgrass, the concentration of five-carbon structural sugars ranged from 183 to 196 mg g^{-1} (Xu *et al*., 2011). Structural xylose concentration in season-long growth of elephantgrass in Florida was similar to that of energycane (135 and 145 mg g^{-1}, respectively) (Fedenko, 2011). For both reed canarygrass and switchgrass, structural five-carbon sugars increased with increasing maturity (147–191 mg g^{-1} in reed canarygrass; 210–253 mg g^{-1} in switchgrass) (Dien *et al*., 2006). This trend was not observed in the present study. Pentose concentrations of 198 and 187 mg g^{-1} from structural carbohydrates have been reported for a mixture of C4 grasses and for a mixture of C3 grasses, respectively, in Minnesota (Gillitzer *et al*., 2013). Although there were statistical differences in pentose concentrations in the present study, unlike hexose concentrations, pentose was relatively constant across harvest managements and entries (avg. of 248 mg g^{-1}) except for 2X-Nov, for which the range was 218–229 mg g^{-1}.

Lignin

Merkeron consistently exhibited the greatest total lignin concentration, and single-harvest per year treatments (1X-Nov and 1X-Dec) showed greater lignin concentration in total aboveground biomass than 2X treatments. Total lignin concentration in the present study is similar to 204–291 mg g^{-1} reported in a previous study for full-season growth of elephantgrass and energycane (Fedenko *et al*., 2013). The results of both studies agree that elephantgrass generally has greater lignin concentration than energycane. The reported lignin concentrations are also similar to those for three switchgrasses, which ranged from 214 to 230 mg g^{-1} for full-season growth (Xu *et al*., 2011). Prior research with alfalfa (*Medicago sativa* L.) and pine (*Pinus* sp.) has shown that greater lignin concentrations decrease pretreatment efficiency relative to lesser concentrations and that even slight changes are sufficient to markedly affect conversion efficiency (Dixon & Chen, 2007; Studer *et al*., 2011). Therefore, greater lignin concentrations in elephantgrass may negatively affect pretreatment and biofuel production.

Nitrogen, phosphorus, and ash

In general, 2X treatments showed greater N concentration compared with 1X treatments. Merkeron had the greatest N concentration in total aboveground biomass. Similar to responses in the present study, harvest frequency of reed canarygrass in Iowa affected N concentration. When biomass was harvested twice per year (June and fall), N concentration was 13.4 (June) and 8.8 mg g^{-1} (fall) compared with 8.3 mg g^{-1} for a single harvest in fall (Tahir *et al*., 2011). In Japan, elephantgrass that was harvested less frequently had lower N concentrations in both leaves and stem than that harvested more frequently (Hsu *et al*., 1990). In the same study, the difference between leaf and stem N concentration increased as harvest interval was extended. When plants reached a height of 1 m, N concentrations were 10.8 mg g^{-1} in leaf vs. 5.0 mg g^{-1} in stem, and when plants were 2 m tall, N concentrations were 8.6 mg g^{-1} in leaf vs. 2.6 mg g^{-1} in stem. Switchgrass biomass N concentration decreased over the season (Madakadze *et al*., 1999); however, N concentration was relatively constant after September at about 5 mg g^{-1}. Miscanthus N concentration decreased until October and then remained constant for the rest of the season (Heaton *et al*., 2009). Similarly, delaying harvest from 1X-Nov to 1X-Dec in the present study did not result in a significant decrease in either leaf or stem N, but 1X-Dec N concentration of total aboveground biomass was slightly less than 1X-Nov because of leaf

 doi: 10.1111/gcbb.12319

senescence and the resultant decrease in leaf : stem ratio in 1X-Dec (Na *et al*., 2015a). When grown with no fertilizer on loamy sands in Georgia, Merkeron elephantgrass had greater overall N concentration than L 79-1002 (3.8 vs. 2.7 mg g^{-1}) (Knoll *et al*., 2012), similar to the pattern of response in the present study, but Singh *et al*. (2015) reported no difference in total aboveground biomass N concentration between L 79-1002 and Merkeron across three sites in Florida.

Similar to N concentration, Merkeron showed the greatest tissue P concentration. The 1X treatments had lesser P concentrations than either of the 2X treatments. Similar results were found for reed canarygrass in Iowa, where P concentration was greater for two harvests per year than for a single harvest (Tahir *et al*., 2011). Switchgrass P concentration was also affected by harvest management, and decreased slightly from the first to second harvest of a two-harvest per year treatment, and was lowest in the fall harvest of a single-harvest per year treatment (1.3, 1.1, and 0.8 mg g^{-1}, respectively) (Guretzky *et al*., 2011). Giant reed and switchgrass P concentrations decreased from October to December in Oklahoma (Kering *et al*., 2012). In switchgrass, leaf N was much greater compared with stem, but leaf and stem P concentrations varied only slightly (Shahandeh *et al*., 2011).

In the present study, harvest management affected ash concentration. For the 1X treatment, total aboveground elephantgrass biomass ash concentration was lower than that for either harvest of the 2X treatment. The results of the present study are similar to those reported previously for Merkeron and L 79-1002. When 4-year data were averaged, Merkeron had greater ash concentration than L 79-1002 when grown with no fertilizer on loamy sands of the coastal plain of Georgia, USA (45.9 vs. 34.4 mg g^{-1}, respectively) (Knoll *et al*., 2012). Reed canarygrass responded differently than elephantgrass and energycane, as ash concentration increased slightly over the season (96, 106, and 107 mg g^{-1} for two harvests per year in June and October, and for one harvest in October, respectively) (Tahir *et al*., 2011). In switchgrass, ash concentration in Iowa peaked in July (71 mg g^{-1}) and decreased until fall after which it remained relatively constant between 43 and 45 mg g^{-1} (Wilson *et al*., 2013). Limited data for plant part ash concentration indicate that leaf was found to have greater ash than stem (Summers *et al*., 2001; Bakker & Elbersen, 2005).

In conclusion, harvest frequency (2X vs. single) significantly affects compositional quality of perennial grasses. A single harvest in fall appears to maximize the concentration of cellulose in total biomass, and delaying harvest from 1X-Nov to 1X-Dec had little impact on most response factors. The exception to this was for extractives and soluble sugars in energycane, which increased significantly from 1X-Nov to 1X-Dec harvest. A relatively greater concentration of soluble sugars in energycane reduced the concentrations of the structural components. A major factor affecting concentration differences due to harvest management was differences in leaf:stem ratio because leaves generally had greater N, P, and ash than stem. Later harvests were associated with lesser leaf percentage in total biomass, which caused N, P, and ash to decrease in 1X-Nov and 1X-Dec relative to 2X treatments. Total aboveground biomass N concentration in Merkeron decreased to a greater extent than the other entries because of greater leaf abscission (Na *et al*., 2015b). The 1X-Nov and 1X-Dec treatments of all entries had lesser concentrations of N and ash, components which can negatively affect some conversion processes, but they had slightly greater lignin concentration than 2X harvests. On the basis of this study results, a single harvest in fall will result in preferred biomass compositional properties, with maximum cellulose concentration of both elephantgrass and energycane. However, unlike energycane, delaying a single harvest of elephantgrass to after a freeze will not compromise cellulose concentration. This study provides needed information on nutrient, carbohydrate, and lignin concentrations and carbohydrate composition in elephantgrass and energycane that will guide decision making regarding biomass harvest frequency and timing for perennial grasses grown for biomass in the southeastern USA.

Acknowledgements

We gratefully acknowledge the assistance of Kenneth Woodard, Dwight Thomas, Miguel Castillo, Kim Mullenix, Nick Krueger, and Marcelo Wallau for their help in data collection and field management. This study is part of the Ph.D. dissertation of the senior author and was directed by Dr. Lynn E. Sollenberger at the University of Florida. This research is funded by the Florida Department of Agriculture and Consumer Services.

References

Adler PR, Sanderson MA, Boateng AA, Weimer PJ, Jung HJ (2006) Biomass yield and biofuel quality of switchgrass harvested in fall or spring. *Agronomy Journal*, **98**, 1518–1525.

Akin DE (2007) Grass lignocellulose: strategies to overcome recalcitrance. *Applied Biochemistry and Biotechnology*, **137**, 3–15.

Bakker RR, Elbersen HW (2005) Managing ash content and quality in herbaceous biomass: an analysis from plant to product. In: *14th European Biomass Conference and Exhibition* (ed. Sjunnesson, L. *et al*.), pp. 210–213. ETA – Renewable Energies, Paris, France.

Bischoff KP, Gravois KA, Reagan TE, Hoy JW, Kimbeng CA, Laborde CM, Hawkins GL (2008) Registration of 'L 79-1002' Sugarcane. *Journal of Plant Registrations*, **2**, 211–217.

Burton GW (1989) Registration of 'Merkeron' napiergrass. *Crop Science*, **29**, 1327–1327.

Casler MD, Boe AR (2003) Cultivar X environment interactions in switchgrass. *Crop Science*, **43**, 2226–2233.

Chaparro CJ, Sollenberger LE (1997) Nutritive value of clipped 'Mott' elephantgrass herbage. *Agronomy Journal*, **89**, 789–793.

 doi: 10.1111/gcbb.12319

Dien BS, Jung HJ, Vogel KP *et al*. (2006) Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass and Bioenergy*, **30**, 880–891.

Dixon RA, Chen F (2007) Lignin modification improves fermentable sugar yields for biofuel production. *Nature Biotechnology*, **25**, 759–761.

Fedenko JR (2011) Biomass yield and composition of potential bioenergy feedstocks. Ph.D dissertation. University of Florida, Florida, USA.

Fedenko JR, Erickson JE, Woodard KR *et al*. (2013) Biomass production and composition of perennial grasses grown for bioenergy in a subtropical climate across Florida, USA. *Bioenergy Research*, **6**, 1082–1093.

Gallaher RN, Weldon CO, Futral JG (1975) An aluminum block digester for plant and soil analysis. *Soil Science Society of America Journal*, **39**, 803–806.

Gillitzer PA, Wyse DL, Sheaffer CC, Taff SJ, Lehman CC (2013) Biomass production potential of grasslands in the oak savanna region of Minnesota, USA. *Bioenergy Research*, **6**, 131–141.

Guretzky JA, Biermacher JT, Cook BJ, Kering MK, Mosali J (2011) Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. *Plant and Soil*, **339**, 69–81.

Hambleton LG (1977) Semiautomated method for simultaneous determination of phosphorus, calcium and crude protein in animal feeds. *Journal-Association of Official Analytical Chemists*, **60**, 845–852.

Hanna WW, Chaparro CJ, Mathews BW, Burns JC, Sollenberger LE, Carpenter JR (2004) Perennial Pennisetums. In: *Warm-Season (C4) Grasses* (eds Moser LE, Burson BL, Sollenberger LE), pp. 503–535. ASA-CSSA-SSSA, Madison, WI.

Heaton EA, Dohleman FG, Long SP (2009) Seasonal nitrogen dynamics of *Miscanthus* × *giganteus* and *Panicum virgatum*. *Global Change Biology-Bioenergy*, **1**, 297–307.

Hoa DT, Man TD, Hau NG (2008) Pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *ASEAN Journal on Science and Technology for Development*, **25**, 341–346.

Hsu F, Hong K, Lee M, Lee K (1990) Effects of cutting height on forage yield, forage and silage quality of napier grasses (written in Chinese with abstract in English). *Journal of the Agricultural Association of China*, **151**, 77–88.

Jakob K, Zhou F, Paterson A (2009) Genetic improvement of C4 grasses as cellulosic biofuel feedstocks. *In Vitro Cellular & Developmental Biology*, **45**, 291–305.

Jung JH, Vermerris W, Gallo M, Fedenko JR, Erickson JE, Altpeter F (2013) RNA interference suppression of lignin biosynthesis increases fermentable sugar yields for biofuel production from field-grown sugarcane. *Plant Biotechnology Journal*, **11**, 709–716.

Kering MK, Butler TJ, Biermacher JT, Guretzky JA (2012) Biomass yield and nutrient removal rates of perennial grasses under nitrogen fertilization. *Bioenergy Research*, **5**, 61–70.

Kim M, Day D (2011) Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. *Journal of Industrial Microbiology and Biotechnology*, **38**, 803–807.

Knoll JE, Anderson WF, Strickland TC, Hubbard RK, Malik R (2012) Low-input production of biomass from perennial grasses in the coastal plain of Georgia, USA. *Bioenergy Research*, **5**, 206–214.

León RG, Gilbert RA, Korndorfer PH, Comstock JC (2010) Selection criteria and performance of energycane clones (*Saccharum* spp. × *S. spontaneum*) for biomass production under tropical and sub-tropical conditions. *Ceiba*, **51**, 11–16.

Lewandowski I, Heinz A (2003) Delayed harvest of miscanthus – influences on biomass quantity and quality and environmental impacts of energy production. *European Journal of Agronomy*, **19**, 45–63.

Lu Y, Mosier NS (2008) Current technologies for fuel ethanol production from lignocellulosic plant biomass. In: *Genetic Improvement of Bioenergy Crops*. (ed. Vermerris W), pp. 161–182. Springer, New York.

Madakadze IC, Stewart K, Peterson PR, Coulman BE, Smith DL (1999) Switchgrass biomass and chemical composition for biofuel in eastern Canada. *Agronomy Journal*, **91**, 696–701.

McKendry P (2002) Energy production from biomass (part 1): overview of biomass. *Bioresource Technology*, **83**, 37–46.

Mylavarapu R, Wright D, Kidder G, Chambliss C (2009) *UF/IFAS Standard Fertilization Recommendations for Agronomic Crops*. Florida Cooperative Extension Service, UF/IFAS, Florida, USA.

Na C, Sollenberger LE, Erickson JE *et al*. (2015a) Management of perennial warm-season bioenergy grasses. II. Seasonal differences in elephantgrass and energycane morphological characteristics affect responses to harvest frequency and timing. *Bioenergy Research*, **8**, 618–626.

Na C, Sollenberger LE, Erickson JE, Woodard KR, Vendramini JM, Silveira M (2015b) Management of perennial warm-season bioenergy grasses. I. Biomass harvested, nutrient removal, and persistence responses of elephantgrass and energycane to harvest frequency and timing. *Bioenergy Research*, **8**, 581–589.

Perez J, Munoz-Dorado J, de la Rubia T, Martinez J (2002) Biodegradation and biological treatments of cellulose, hemicellulose and lignin. *International Microbiology*, **5**, 53–63.

SAS Institute (2008) *SAS Online DOC 9.2*. SAS Inst., Cary, NC.

Shahandeh H, Chou CY, Hons FM, Hussey MA (2011) Nutrient partitioning and carbon and nitrogen mineralization of switchgrass plant parts. *Communications in Soil Science and Plant Analysis*, **42**, 599–615.

Singh MP, Erickson JE, Sollenberger LE, Woodard KR, Vendramini JM, Gilbert RA (2015) Mineral composition and removal of six perennial grasses grown for bioenergy. *Agronomy Journal*, **107**, 466–474.

Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D (2008a) *Determination of Extractives in Biomass; Laboratory Analytical Procedure (LAP)*. National Renewable Energy Laboratory, Colorado, USA.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008b) *Determination of Structural Carbohydrates and Lignin in Biomass; Laboratory Analytical Procedure (LAP)*. National Renewable Energy Laboratory, Colorado, USA.

Smith R, Slater FM (2011) Mobilization of minerals and moisture loss during senescence of the energy crops *Miscanthus* × *giganteus*, *Arundo donax* and *Phalaris arundinacea* in Wales, UK. *Global Change Biology-Bioenergy*, **3**, 148–157.

Sollenberger LE, Woodard KR, Vendramini JM *et al*. (2014) Invasive populations of elephantgrass differ in morphological and growth characteristics from clones selected for biomass production. *Bioenergy Research*, **7**, 1382–1391.

Studer MH, Demartini JD, Davis MF *et al*. (2011) Lignin content in natural Populus variants affects sugar release. *Proceedings of the National Academy of Sciences*, **108**, 6300–6305.

Summers ME, Jenkins BM, Hyde PR, Williams JF, Scardacci SC (2001) *Properties of Rice Straw as Influenced by Variety, Season and Location*. American Society of Agricultural Engineers Annual International Meeting, California, USA.

Tahir MH, Casler MD, Moore KJ, Brummer EC (2011) Biomass yield and quality of reed canarygrass under five harvest management systems for bioenergy production. *Bioenergy Research*, **4**, 111–119.

Tew TL, Miller JD, Dufrene EO *et al*. (2011) Registration of 'HoCP 91-552' sugarcane. *Journal of Plant Registrations*, **5**, 181–190.

Thammasouk K, Tandjo D, Penner MH (1997) Influence of extractives on the analysis of herbaceous biomass. *Journal of Agricultural and Food Chemistry*, **45**, 437–443.

Tran A, Chambers R (1986) Lignin and extractives derived inhibitors in the 2,3-butanediol fermentation of mannose-rich prehydrolysates. *Applied Microbiology and Biotechnology*, **23**, 191–197.

Vermerris W (2008) Composition and biosynthesis of lignocellulosic biomass. In: *Genetic Improvement of Bioenergy Crops* (ed. Vermerris W), pp. 89–142. Springer, New York, USA.

Waramit N, Moore KJ, Heggenstaller AH (2011) Composition of native warm-season grasses for bioenergy production in response to nitrogen fertilization rate and harvest date. *Agronomy Journal*, **103**, 655–662.

Wilson DM, Dalluge DL, Rover M, Heaton EA, Brown RC (2013) Crop management impacts biofuel quality: influence of switchgrass harvest time on yield, nitrogen and ash of fast pyrolysis products. *Bioenergy Research*, **6**, 103–113.

Woodard KR, Prine GM (1993) Regional performance of tall tropical bunchgrasses in the southeastern USA. *Bioenergy Research*, **5**, 3–21.

Xu J, Chen Y, Cheng J, Sharma-Shivappa RR, Burns JC (2011) Delignification of switchgrass cultivars for bioethanol production. *Bioresource Technology*, **6**, 707–720.

Zhang K, Agrawal M, Harper J, Chen R, Koros WJ (2011) Removal of the fermentation inhibitor, furfural, using activated carbon in cellulosic-ethanol production. *Industrial & Engineering Chemistry Research*, **50**, 14055–14060.